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FY 1992 ANNUAL REPORT

**VETERINARY RESOURCES PROGRAM
NATIONAL CENTER FOR RESEARCH RESOURCES
NATIONAL INSTITUTES OF HEALTH**



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FY 1992 - VRP ANNUAL REPORT

VETERINARY RESOURCES PROGRAM
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NATIONAL INSTITUTES OF HEALTH

MARLENE N. COLE, D.V.M.
DIRECTOR

VETERINARY RESEARCH PROJECTS (Z01 RR)

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-RR-00001-22 VR

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Animal Model Development

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Carl T. Hansen, Ph.D.

Geneticist

GRS, SSB, VRP, NCRR

COOPERATING UNITS (if any)

LAB/BRANCH

Veterinary Resources Program

SECTION

Genetic Resource Section, SSB

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The project is designed to meet a demand by the research community for small animal models for the study of those human diseases with major health implications. Developmental efforts have focused on rat models of rheumatoid arthritis, insulin independent diabetes mellitus and chemical addictions. In mice, the primary effort has been directed toward developing models of immunodeficiency which are beginning to find broad usage in those areas of biomedical research in which the immune system plays an important role. Other areas which are either being planned or initiated consist of characterizing selected closed breeding groups of rats for a variety of behavioral traits in preparation for studies of the molecular basis for these traits.

Objective:

To develop a resource of small research animals supporting the biomedical research community.

Methodology:

The focus for this project is to develop a resource of genetically defined, small research animals which meets the ongoing and future needs of the research community by the process of selective choice of individuals exhibiting specific traits to be parents for the succeeding generations. This assumes that the trait or traits in question have at least a partial genetic component.

Major Findings:

This project impacts a broad spectrum of the research community. Although a particular project may be undertaken for a specific research area, the results often may have broader ramifications than originally intended.

Immunocompromised Mice:

Mice falling into this category were originally developed in response to shortcomings observed in mice carrying single gene mutations affecting the immune system. The approach has been to combine, on a variety of genetic backgrounds, mutations which are known to affect different components of the immune system. The original models represented a combination of three genes affecting different components of the immune system. More recently, this has been modified to include a fourth mutation again affecting a different component of the immune system.

Rat Models for Insulin Independent Diabetes Mellitus (IIDM):

Continued studies with the SHR/N-cp (corpulent) congenic strain increasingly demonstrate its utility as a model for IIDM in that many of its features are similar to that found in humans. Comparative studies with additional rat strains suggest a relationship between hypertension and this disease. Also, it has been found that at one genetic background, stressing the animals with a high sucrose diet yields a situation of severe blood vessel wall damage similar to that seen in patients with malignant forms of hypertension.

Models in Rats for the Study of Rheumatoid Arthritis:

These studies continue but with a shift toward elucidating the molecular basis of the disease. DNA probes are being developed specifically to define the molecular nature of this disease. As an extension of this study, a project to define genetic polymorphisms in a broader spectrum of inbred strains has been undertaken. The ultimate goal is to develop a genetic linkage map in the rat. One result of these studies has been establishing the linkage map for the nude (rnu) gene in the rat. In addition, evidence has been found that the LER rat strain is, in reality, a hybrid between the LEW and BUF inbred rat strains.

Proposed Course:

Continuation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-RR-00071-08 VR

3

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Genetic Profiles for Inbred Laboratory Rodents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

James S. Crowell, Ph.D.

Geneticist

LSS, SSB, VRP, NCRR

COOPERATING UNITS (if any)

LAB/BRANCH

Veterinary Resources Program

SECTION

Laboratory Sciences Section, SSB

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.1

PROFESSIONAL:

0.05

OTHER:

0.05

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project was designed to identify and locate on the chromosomes of inbred rodents specific inherited characteristics. In addition to being utilized in a wide variety of biomedical research, this knowledge can substantially increase the ability of the Genetic Monitoring Unit to perform its basic mission of genetic quality assurance. Major areas of interest include the characterization of genetic traits by biochemical, immunological, and molecular biological techniques as well as chromosome mapping by standard genetic analysis. This genetic characterization is used to explore new animal models of pathogenesis.

Objective:

To identify inheritable traits in inbred rodents which can be utilized in a genetic quality assurance program.

Methodology:

A variety of biochemical techniques, predominantly electrophoretic and spectrophotometric procedures, were used to analyze genetically controlled variations in proteins. Selective breeding of specific strains was accomplished to elucidate the genetic control of this process.

Major Findings:

Genetic profiles have been established for the inbred strains of rodents maintained in the NIH Animal Genetic Resource (NIHAGR).

Significance:

These profiles are utilized on a routine basis by the Genetic Monitoring Unit to ensure the genetic integrity of animals bred in the NIHAGR. The development of more extensive genetic profiles has increased the ability of the Unit to detect problems in the propagation of genetically defined animals. These findings are also of interest to workers in a wide variety of fields when selecting an animal model for a specific research problem.

Proposed Course:

Completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-RR-00077-08 VR

5

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of an Embryo Cryopreservation Program in Laboratory Animals

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Patricia M. Schmidt, M.S.	Physiologist	GRS, SSB, VRP, NCRR
Carl T. Hansen, Ph.D.	Geneticist	GRS, SSB, VRP, NCRR
Victoria M. Hollifield, D.V.M.	Veterinarian	GRS, SSB, VRP, NCRR
Xi Lin, D.D.S.	Fogarty Fellow	GRS, SSB, VRP, NCRR
William F. Rall, Ph.D.	Guest Researcher	VRP, NCRR

COOPERATING UNITS (if any)

Department of Animal Health, National Zoological Park, Smithsonian Institution
(D.E. Wildt)

LAB/BRANCH

Veterinary Resources Program

SECTION

Genetic Resource Section, SSB

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

2.0

OTHER:

1.0

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

The NIH Animal Genetic Resource (NIHAGR) maintains an array of animal model species and, within species, a large number of invaluable genotypes used in biomedical research. For this reason, establishing an embryo cryobank is essential. Cryopreservation and storage of preimplantation stage embryos in liquid nitrogen offers a convenient and economical method for preserving strains and stocks of rodents and rabbits for indefinite periods of time. In addition to stock maintenance, embryo cryopreservation offers other important benefits. First, a bank of cryopreserved embryos provides insurance against the loss of genetic resources due to disease, breeding failure, natural disaster or inadvertent genetic contamination. Second, unavoidable genetic changes due to genetic drift can be minimized, thereby facilitating the development of "Type Cultures" of defined, inbred mouse strains for periodic restandardization of foundation breeding colonies. Third, the international distribution of breeding nuclei in the form of cryopreserved embryos is simpler, less expensive, more humane and easier than the transport of animals. Finally, embryo collection, cryopreservation and transfer offer effective and inexpensive methods for interrupting the vertical transmission of many murine diseases.

Objective:

The objective is to study the development and cryobiology of embryos and gametes from laboratory animal species for the purpose of improving long-term cryostorage of valuable genetic material managed within the NIH Animal Genetic Resource (NIHAGR).

Methodology:

Pre-implantation stage embryos were collected from hormonally-treated or natural estrus females following euthanasia or at surgery. Embryos were frozen or vitrified in medium containing cryoprotectant. Following storage in liquid nitrogen (-196°C), straws containing embryos were warmed and the cryoprotectant removed using a sucrose dilution procedure. Diluted embryos were assessed in vitro or in vivo. Embryo stage of development and quality were observed daily to determine in vitro survival for embryos in culture. In vivo evaluation involved surgical transfer of embryos into synchronized, pseudopregnant recipients. Recipients were monitored for pregnancy and allowed to go to term. If embryos were intended as additions to the program cryobank, individual animal pedigree records were maintained on the Genetic Resource Section animal database system.

Major Findings:

Since 1984, 215,464 mouse embryos were collected, 59,753 of these embryos were permanently banked and the remainder were used in comparative biological studies. To date, a minimum of 1,000 non-pedigreed embryos have been stored from each of 38 NIHAGR genotypes. During FY 1992, over 17,000 embryos were collected from 66 mouse strains. More than 7,600 embryos were cryopreserved including 5,871 embryos from these 66 genotypes that were added to the embryo bank. Pedigreed embryos banked now total approximately 11,000. All embryos banked this year were collected from pedigreed donors.

Changes in the software supporting the barrier room foundation colonies (FC) benefited the mouse cryobanking efforts. Modification increased program efficiency by: 1) permitting multiple FC colonies to be maintained concurrently to allow banking of these genetically ideal embryos from animals in 14G/117; 2) allowing these FC animals to be used instead of the pedigreed expansion colony females used to date; and 3) approaching the point where this software can integrate with program records to track the location and use of the pedigreed FC donors through embryo cryostorage and subsequent rederivation of FC animals at a later date. Additionally, FC females were prepared for embryo recovery in a barrier room and then sent to a laboratory.

Another effort was directed at modifying the hormonal regimen (FMSG and hCG) used to superovulate rabbits. Presumed overstimulation interfered with or prevented attempts to recover embryos from some females. Lower hormone dosages and the use of a different gonadotropin (FSH-P) reduced the incidence of reproductive tract hemorrhage at surgery but did not reduce the number of embryos recovered. Modification of a standard FSH-P regimen appeared to improve the condition of the donor reproductive tract at surgery. Administering FSH-P at 24 hour, rather than at 12 hour, intervals did not compromise ovulation or embryo recovery rates. This modified regimen appears valuable for stimulating follicle development in females (1) suspected to be too old to mate successfully for the first time and (2) used as second-time embryo donors. Perhaps this approach would be suitable for older females as a method of "rescuing" genetic material from retired breeders prior to euthanasia.

The use of a luteinizing hormone-releasing analog to synchronize rat genotypes was evaluated to: 1) provide females for embryo collection following a natural estrus; 2) provide a starting point for evaluating superovulation regimens previously reported to be successful in rats (PMSG/hCG and FSH-P osmotic pumps); 3) provide recipients for embryo transfer procedures; and 4) provide timed-pregnant females for Cesarean section of litters. The latter is especially important for improving the success rate for introducing new genotypes into the NIHAGR without relying upon the "random cyclicity" of the females.

Significance:

The concerns surrounding conservation of valuable germ plasm are just developing, as historically these problems have not been at the forefront of resource management and biomedical research issues. Because of the wide range of species and genotypes representing several genetic backgrounds maintained in the NIHAGR, this is an important issue. Gamete cryopreservation presents a convenient and economical method to maintain the gene pool while limiting the impact of genetic drift on these colonies.

Proposed Course:

Continuation.

Publications:

Wildt DE, Donoghue AM, Johnston LA, Schmidt PM, Howard JG. Species and genetic effects on the utility of biotechnology for conservation. Symposium Zool Soc London 1992;64:45-61.

Schiewe MC, Hansen CT, Schmidt PM. Lack of antibody specificity by mouse trophoctoderm during immunosurgery. Theriogenology 1992;38:21-32.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-RR-00086-03 VR
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Characterization of a Feline Pneumonia Outbreak		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Jennifer A. Burris, D.V.M.	Veterinary Pathologist	LSS, SSB, VRP, NCRR
Elaine Woo, B.S.	Biologist	LSS, SSB, VRP, NCRR
Vikki S. Kinsey, B.S.	Biologist, Microbiologist	LSS, SSB, VRP, NCRR
Debra Bower, B.A.	Microbiologist	LSS, SSB, VRP, NCRR
COOPERATING UNITS (if any)		
LAB/BRANCH Veterinary Resources Program		
SECTION Laboratory Sciences Section, SSB		
INSTITUTE AND LOCATION NCRR, NIH, Bethesda, MD 20892		
TOTAL STAFF YEARS: 0.1	PROFESSIONAL: 0.06	OTHER: 0.04
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) During January, 1990, five cats from a recently imported colony of 48 cats at the NIHAC developed fatal respiratory tract infections. Bacterial cultures of lung tissues yielded CDC Group EF-4 from four cats. Although sporadic cases of feline pneumonia associated with this poorly defined bacterial group have been reported, this outbreak within a single colony is unique. This study served: (1) to further characterize, by biochemical reactions and SDS PAGE analysis, the case-associated bacterial isolates by comparing them with control EF-4 strains obtained from other sources; (2) to assess the pathology of the lesions associated with EF-4 infection; and (3) to review the medical histories and clinicopathologic findings from cats which died of EF-4 pneumonia and from cats which died from other causes to determine if there were any underlying diseases which contributed to the pneumonia outbreak in this colony. SDS PAGE analysis showed that NIH cat EF-4 bacteria isolates share an identical protein profile, which is unique from profiles of other control EF-4 strains. Necropsy findings include bronchopneumonia (necrosuppurative to pyogranulomatous) and pleuritis (fibrinous to fibropurulent) due to EF-4 bacterial infections. Feline leukemia virus infections, along with shipping stress, may have contributed toward this outbreak of EF-4 pneumonia.		

Objectives:

1) To determine if Group EF-4 strains obtained from feline lung tissues during an outbreak of respiratory disease are related and if they can be differentiated from other Group EF-4 strains not associated with the outbreak; 2) To document the pathologic processes caused by Group EF-4 in this outbreak; and 3) To review the clinical and pathologic data for the presence of immunosuppressive agents and/or underlying diseases in this colony.

Methodology:

Bacterial Identification:

Lung tissues collected at necropsy were inoculated onto 5% sheep blood trypticase soy agar, MacConkey agar, and trypticase soy broth. Cultures were incubated under aerobic conditions at 36°C. Small dry colonies with a tan to yellow pigment were observed on 5% sheep blood agar at 48 to 72 hours of incubation. These colonies consisted of oxidase positive, catalase positive, gram negative coccobacilli that failed to ferment most carbohydrates. Confirmatory tests, performed at both the NIH and the Centers for Disease Control, identified these isolates as Group EF-4.

SDS PAGE:

EF-4 strains were inoculated onto 5% sheep blood trypticase soy agar plates and incubated for 24 hours at 36°C. Bacteria were then suspended in sterile saline to match a MacFarland turbidity standard #6 (1.8×10^9 bacteria/mL). Aliquots of the standard suspensions containing 1.5 mL were concentrated by centrifugation and resuspended to a final volume of 50 μ L, to which 200 μ L of SDS PAGE solubilization buffer was added. Samples containing 25 μ L were then analyzed by SDS PAGE using 5% stacking gels and 12.5% separating gels. Electrophoresis gels and buffers were constructed using standard protocols.

Clinicopathologic Review and Correlations:

Animal health records, including clinical pathology results and histopathologic findings, were reviewed, assessed and correlated from all fatalities (including EF-4 pneumonias) in this colony.

Major Findings:

SDS PAGE analysis showed all of the four cat isolates to share an identical protein profile, which is unique from profiles of other control EF-4 strains. Necropsy findings included bronchopneumonia (necrosuppurative to pyogranulomatous), pleuritis (fibrinous to fibropurulent), hepatic lipidosis, rhinitis, conjunctivitis, gingivitis, enteritis, splenic lymphoid atrophy, bacterial septicemia, pyometria, and cardiomyopathy. It has been concluded that the EF-4 bacterial infection, feline virus infection, and the stress associated with shipping contributed toward the outbreak of EF-4 pneumonia.

Significance:

Feline pneumonias associated with EF-4 bacteria are extremely rare events. The studies outlined in this project should expand our knowledge of the epidemiology and microbiology of this outbreak.

Proposed Course:

Completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-RR-00087-03 VR

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Serum Cholesterol and Triglyceride Values of the NIH WHHL Rabbit Colony

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Dennis E. Barnard, M.S.

Biologist

LSS, SSB, VRP, NCRR

Victoria M. Hollifield, D.V.M.

Veterinarian

GRS, SSB, VRP, NCRR

Carl T. Hansen, Ph.D.

Geneticist

GRS, SSB, VRP, NCRR

COOPERATING UNITS (if any)

LAB/BRANCH

Veterinary Resources Program

SECTION

Laboratory Sciences Section, SSB

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Veterinary Resources Program, National Center for Research Resources, has established a Watanabe heritable hyperlipidemic (WHHL) rabbit breeding colony as a resource for investigators requiring this animal model. Total serum cholesterol values range from 310 to 1251 mg/dl with a mean of 828 mg/dl. The low density lipoprotein (LDL) cholesterol values range from 70 to 1185 mg/dl with a mean of 630 mg/dl. Serum triglyceride values range from 107 to 959 mg/dl with a mean of 394 mg/dl. There appears to be a correlation between serum triglyceride concentration and the sex of the rabbit. The mean male and female triglyceride concentrations were 510 mg/dl and 309 mg/dl, respectively.

Objective:

The purpose of this project is to characterize the serum cholesterol and triglyceride values of the NIH WHHL rabbit colony.

Methodology:

Blood was collected from WHHL rabbits at eight weeks, three months, seven months, and twelve months of age. The serums were analyzed for lipoprotein cholesterol and triglycerides. A computer program has been developed to track pedigrees of the WHHL rabbits, making it possible to evaluate the influence of genotype on cholesterol and triglyceride values.

Major Findings:

Total serum cholesterol values for the NIH WHHL rabbit colony ranged from 310 to 1251 mg/dl with a mean of 828 mg/dl. The LDL cholesterol values ranged from 70 to 1185 mg/dl with a mean of 630 mg/dl. Thus, 80% of the total cholesterol was composed of LDL cholesterol. There was a substantial decrease from 885 to 576 mg/dl in serum total cholesterol between the ages of eight weeks and one year--the most significant decrease occurring between 15 and 25 weeks. Differences in total cholesterol concentrations were not related to sex. Serum triglyceride values ranged from 107 to 959 mg/dl with a mean of 394 mg/dl. There was an initial increase in triglyceride concentration from 367 to 565 mg/dl between two months and three months of age. The serum triglyceride concentration was influenced by the sex of the rabbit. The mean male and female serum triglyceride concentrations were 510 mg/dl and 309 mg/dl, respectively.

Significance:

It appears that serum cholesterol and triglyceride values are influenced by age and sex, respectively.

Proposed Course:

Continuation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-RR-00088-03 VR

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Experimental Reconstitution of Embryonic Trophoctoderm and Inner Cell Mass

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Mitchel C. Schiewe, Ph.D.

NRC Research Associate

GRS, SSB, VRP, NCRR

Patricia M. Schmidt, M.S.

Physiologist

GRS, SSB, VRP, NCRR

Carl T. Hansen, Ph.D.

Geneticist

GRS, SSB, VRP, NCRR

COOPERATING UNITS (if any)

LAB/BRANCH

Veterinary Resources Program

SECTION

Genetic Resource Section, SSB

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.2

PROFESSIONAL:

1.2

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Developing practical and efficient procedures for isolating the precursor placental (trophoctoderm) and fetal (inner cell mass) cellular components of the developing mouse blastocyst was the principal aim of this project. Our specific objective was to successfully reconstruct the blastocyst, a process referred to as blastocyst reconstitution, in an attempt to avoid fetal rejections associated with interspecies embryo transfer. By injecting the inner cell mass of one species (or genotype) into the trophoblast (trophoctoderm vesicle) of the recipient genotype, it is sometimes possible to eliminate fetal loss associated with immunological rejections and, thus, increase the reproductive potential of a genetically valuable animal (e.g., rare animal model or endangered species). These experiments focused on specific questions that involved the development of effective methods for isolating and then recombining inner cell masses and zona pellucida-enclosed trophoctoderm vesicles.

Objectives:

Using mouse embryos, the objectives were to: 1) develop a reliable technique for producing zona pellucida-intact trophectodermal vesicles (TEVs); 2) establish an effective blastocyst injection procedure to allow for the penetration of a large diameter injection pipette; and 3) determine the ability of the inner cell mass (ICM) to support foreign TE by producing live, non-chimeric offspring using genetically-distinct mouse strains or species.

Methodology:

Precompacted 8-cell embryos were used to produce blastocysts without a viable ICM. Group 1 embryos were exposed to Ca^{++} -free medium containing EGTA and trypsin for 30 minute intervals during in vitro culture. Group 2 embryos were cultured in medium containing cytochalasin D. Group 3 embryos were placed in medium + [^3H]methyl-thymidine for 24 hours. Controls included non-treated blastocysts and mechanically-punctured blastocysts dissected free of ICMs. Cellular viability and TEV purity were assessed subjectively by examining monolayers for the presence of ICM cells during 1 week in vitro. In vivo evaluation of TEV purity was performed following transfer to pseudopregnant recipients. Three days later, the embryonic vesicles were recovered, fixed and embedded for histological examination. Another test of in vivo viability of [^3H]thymidine-treated TEVs was microinjection of a reciprocal strain ICM and subsequent transfer of the reconstructed blastocysts. Non-treated blastocysts and mechanically-produced TEVs alone were control transfers.

Major Findings:

Both chemical treatments were ineffective at inhibiting normal differentiation without significantly reducing continued embryo development. Using [^3H]thymidine, however, was an effective method that selectively interfered with replication of ICM. The 100 nCi/ml treatment was optimal for forming zona-enclosed TEVs. In vitro, these latter embryos showed high rates of development. Subsequently, approximately 43% of the later-staged embryos formed monolayers without ICM cells, but 56% showed remnant ICM tissue. Most controls (93%) showed normal monolayer proliferation. At higher concentrations more monolayers were ICM-free, but overall embryo viability was reduced. In vivo studies assessed the viability of the trophoblast as well as any remnant ICM cells. Some vesicles were observed as late as 84 hours post-transfer, suggesting that the developmental capacity of the trophoblast is not destroyed by irradiation.

A blastocyst injection procedure was established. Maintaining blastocysts or zona-intact TEVs at 5°C (~3 hrs.) reduced the elasticity of TE membranes sufficiently to allow rapid penetration of large injection pipettes. Sham micromanipulation trials determined that this was not detrimental to embryo viability. Blastocyst reconstitution was successful using the 1-step microinjection approach under in vitro conditions.

Significance:

Isolation of embryonic components of the developing blastocyst provides a tool for maximizing the success of interspecies pregnancies. Using the trophoblast of the recipient species, it may be possible to offset fetal loss associated

with immunological rejection of the foreign placenta, while supporting the continued development of the ICM of another species. This blastocyst reconstitution procedure has potential therapeutic use to enhance reproductive efficiency of NIHAGR genotypes with low implantation success.

Proposed Course:

Completed.

Publications:

Schiewe MC, Bush M, Phillips LG, Citino S, Wildt DE. Comparative aspects of estrous synchronization, ovulation induction and embryo cryopreservation in the scimitar-horned oryx, bongo, eland and greater kudu. *J Exp Zool* 1991;58:75-88.

Schiewe MC, Bush M, de Vos V, Wildt DE. Semen characteristics, sperm freezing and endocrine profiles in free-ranging wildebeest (*Connochaetes taurinus*) and greater kudu (*Tragelaphus strepsiceros*). *J Zoo Wildl Med* 1991;22:58-72.

Schiewe MC, Fitz TA, Brown JL, Stuart LD, Wildt DE. Premature luteal regression in superovulated sheep: Relationship to estrous synchronization, circulating hormones, luteinizing hormone/prostaglandin F_{2a} receptors and luteal progesterone content. *J Reprod Fert* 1991;93:19-30.

Schiewe MC, Stuart LD, Wildt DE. Ovine embryo cryopreservation: Analysis of cryoprotectant cooling rate and *in situ* straw dilution using conventional freezing or vitrification. *Theriogenology* 1991;36:279-293.

Schiewe MC, Hansen CT, Schmidt PM. Lack of antibody specificity by mouse trophoblast during immunosurgery. *Theriogenology* 1992;38:21-32.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-RR-00089-03 VR
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Excreted Steroids in Primate Feces		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Samuel K. Wasser, Ph.D. Research Fellow Smithsonian Institution Janice L. Southers, D.V.M. Veterinarian PS, RAB, VRP, NCRR		
COOPERATING UNITS (if any) Department of Animal Health, National Zoological Park, Smithsonian Institution (D.E. Wildt)		
LAB/BRANCH Veterinary Resources Program		
SECTION Primate Section, RAB		
INSTITUTE AND LOCATION NCRR, NIH, Bethesda, MD 20892		
TOTAL STAFF YEARS: 1.2	PROFESSIONAL: 1.0	OTHER: 0.2
CHECK APPROPRIATE BOXES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) This study is a continuation of work to validate noninvasive techniques to measure biologically meaningful levels of reproductive and adrenal steroid hormones in feces of captive-maintained baboons. Results will serve to establish the general validity of these techniques for use on a variety of species in captivity and the field. Captive studies have: 1) identified fecal metabolites of estradiol (E2), progesterone (P4), cortisol (C), dehydroepiandrosterone (DHEA), and testosterone (T), as well as respective excretion rates after radiolabel infusion; 2) developed indices of nonspecific steroid excretion rate, particularly those associated with GI transit time; 3) tested the influence of dietary fiber on fecal steroid excretion rate, and the potential of eliminating existing effects using nonspecific excretion rate indices; 4) correlated longitudinal fecal steroid excretion profile with blood hormone secretory patterns and visual markers (e.g., menses and sex skin turgescence) during natural reproductive cycles. These excreted steroid techniques are likely to provide an important noninvasive tool for interrelating reproductive function and adrenal activation with naturally and/or artificially occurring socio-ecological events. The majority of this work is near completion. One paper summarizing work from objectives 2-4 recently has been submitted for publication in the <u>Journal of Reproduction and Fertility</u>.		

Objectives:

1) Develop a method for rapid extraction of gonadal and adrenal steroids from feces; 2) determine the diversity of biologically meaningful steroids that can be measured in feces; 3) develop means to control for diet-related changes in steroid excretion rate; and 4) show that these techniques work under variable dietary conditions in the lab.

Methodology:

Hormone excretion rate was examined by injecting animals with a labeled-marker hormone, collecting all urine and feces until the labeled hormone has been completely shed. Excretion rate was measured by monitoring change in the concentration of the labeled hormone appearing in feces over time. Chromatography (HPLC) was used on the material to determine whether the hormone was shed intact or as some metabolite. With this technique we have increased the sensitivity of existing measures of estradiol (E2) and progesterone (P4) and are completing development of assays for cortisol, testosterone, and dehydroepiandrosterone (DHEA). The radiolabel infusions were conducted on menstruating subjects, maintained on each of 3 different dietary regimens: low, medium and high fiber diets. This procedure determined how dietary fiber affected excretion rate of each steroid being measured. Blood sampling occurred 3 days/week, in-between radiolabel infusion studies; urine and feces were collected 5 days/week for the duration. Several markers of steroid excretion rate were examined to determine which technique best controlled for hormone excretion rate in feces--i.e., resulted in the closest correspondence between naturally occurring serum and fecal (and urine and fecal) hormone levels.

Major Findings:

The number of steps required to extract steroids from fecal samples was significantly reduced. This reduction increased the number of samples that can be analyzed from 20 to over 100 per week, as well as improved extraction efficiencies by over 15%. Estradiol and estrone were identified as the dominant estrogens in feces. The dominant progestogens were pregnenolone, progesterone and pregnanediol. It was noted that expressing hormone concentrations on a per gram dry weight basis controlled, to a large degree, the effects of diet. A cholesterol metabolite, cholestanone, was identified which served as an excellent index of steroid excretion rate. However, it was concluded that use of this technique was necessary only under conditions of extreme variation in dietary fiber. Perhaps most exciting was the isolation and purification of the four major cortisol metabolites in feces. These have been sent out for identification using GC mass-spectrophotometry. Once identified, this antigen will be injected in rabbits to develop an antibody for the dominant fecal cortisol metabolite.

Significance:

These techniques have a number of uses for captive management. They should be particularly useful in animal well-being and captive propagation studies, providing noninvasive physiologic indices of stress and reproductive performance. These techniques also will be useful in studies designed to evaluate the physiologic consequences of particular stressors; such studies previously have suffered from the potential confound of stress associated with anesthesia and/or blood withdrawal. Other uses of these techniques include: pregnancy confirmation without the need for palpation or ultrasound, thereby reducing the risk of diagnostic-induced abortion. By controlling for

excretion rate, these measures will remain useful when animals are required to be maintained on specific diets for experimental or clinical purposes. These techniques also will have value for field work, providing for the first time a physiologic window into the lives of free-ranging animals. Such techniques will enable researchers to examine how naturalistic stressors impact the physiologic and particularly reproductive condition. Until now, such studies have been restricted to captivity, where stressors often are necessarily different from those experienced in the natural world.

Proposed Course:

The project is temporarily on hold until the cortisol metabolite antibody has been produced. At this time, the fecal samples will be analyzed for cortisol metabolites and correlated with cortisol concentrations found in their corresponding serum samples. If close correspondence exists between serum and fecal cortisols, the study will be terminated. Otherwise, the final stage of this study will begin--conducting an ACTH challenge on 2-4 females, collecting all feces for the next 3 days. That will constitute the last study to be conducted on this project. Results of the radiolabeled infusion studies are being compiled for publication and will be submitted to the Journal of Reproduction and Fertility. Results of the cortisol studies will be compiled for publication as soon as the remaining data are available.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-RR-00090-03 VR

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of the WHHL Rabbit Lipoprotein Phenotypes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Dennis E. Barnard, M.S. Biologist LSS, SSB, VRP, NCRR

Victoria M. Hollifield, D.V.M. Veterinarian GRS, SSB, VRP, NCRR

James S. Crowell, Ph.D. Geneticist LSS, SSB, VRP, NCRR

Carl T. Hansen, Ph.D. Geneticist GRS, SSB, VRP, NCRR

COOPERATING UNITS (if any)

Clinical Pathology Department, CC

LAB/BRANCH

Veterinary Resources Program

SECTION

Laboratory Sciences Section, SSB

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Veterinary Resources Program, National Center for Research Resources, has established a Watanabe hyperlipidemic (WHHL) rabbit breeding colony as a resource for investigators. Watanabe et al reported that there are two types of WHHL rabbits--those with early development and high incidence of coronary atherosclerosis (type 1) and those with late development and low incidence of coronary atherosclerosis (type 2). It has also been shown that either type of rabbit can be selectively bred to produce progeny exhibiting the phenotype of interest. Recently, it was reported that these two types of WHHL rabbits can be differentiated by their lipoprotein phenotypes. Electrophoretic analysis of 14 rabbits from the NIH WHHL rabbit colony showed that there are two lipoprotein phenotypes: one with an increased density of beta-lipoprotein and another with a beta-doublet. These phenotypes are similar to those reported for the two types of WHHL rabbits.

Objectives:

The purpose of this study is to characterize the lipoprotein phenotypes in the NIH WHHL rabbit colony so that the two types of WHHL rabbits can be identified and identify the apoprotein E phenotypes of the WHHL rabbits.

Methodology:

Rabbits were fasted overnight and blood was collected in EDTA vacutainers the next morning. Agarose gel electrophoresis was used to identify the lipoprotein phenotypes.

Major Findings:

Two lipoprotein phenotypes were identified: one with an increased density of beta-lipoprotein and the other with a beta-doublet. These phenotypes are similar to those reported in the two types of WHHL rabbits. The results of this study indicate that the WHHL rabbits with the beta-doublet phenotype and the type 1 WHHL rabbits have similar VLDL characteristics.

Significance:

The two lipoprotein phenotypes indicate that both the rabbit strain with the high incidence of coronary atherosclerosis and those with a low incidence may be present in the colony. Determination of the lipoprotein phenotypes will enable the Genetic Resource Section to selectively breed the rabbits, providing a more defined WHHL rabbit. A comparison of the beta-doublet lipoprotein phenotype observed in NIH WHHL rabbits and the phenotype reported for type 1 WHHL rabbits with human lipoprotein phenotypes indicates there is a resemblance to type III hyperlipoproteinemia. Type III hyperlipoproteinemia is the result of an apoprotein E defect. Therefore, some WHHL may have an apoprotein E defect as well as an LDL receptor defect. This would explain the high incidence of coronary atherosclerosis in the type 1 WHHL rabbits.

Proposed Course:

Continuation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-RR-00095-02 VR

21

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathogenesis of Ophidian Paramyxovirus Infection in Brown Tree Snakes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Elaine Woo, B.S. Biologist LSS, SSB, VRP, NCRR

Thomas W. Spencer, B.S. Microbiologist LSS, SSB, VRP, NCRR

COOPERATING UNITS (if any)

Department of Pathology, National Zoological Park, Smithsonian Institution
(D. Nichols).

LAB/BRANCH

Veterinary Resources Program

SECTION

Laboratory Sciences Section, SSB

INSTITUTE AND LOCATION

NCRR, NIH; Bethesda, MD 20892

TOTAL STAFF YEARS:

0.2

PROFESSIONAL:

0.1

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This is an ongoing study to determine whether the brown tree snake (Boiga irregularis) is susceptible to infection with ophidian paramyxovirus. The pathogenesis of the viral infection in brown tree snakes is being studied. Virus-induced lesions have not yet been detected in the brown tree snake. Paramyxovirus has been isolated from infected brown tree snake tissues through cell culture and identified by electron microscopy. The National Zoo is continuing snake infection research and the LSS is continuing virus isolation research.

Objectives:

To experimentally infect two species of snakes, the brown tree snake (*Boiga irregularis*) and the prairie kingsnake (*Lampropeltis calligaster*) and compare/contrast the pathogenesis of the infection in these species.

Methodology:

Four brown tree snakes and two prairie kingsnakes were inoculated intratracheally and per os with ophidian paramyxovirus obtained from rattlesnake tissues collected during a recent outbreak of fatal paramyxovirus in snakes at a large U.S. zoo.

The snakes are being individually housed in Horsfal isolation units and are closely monitored for signs of illness. Blood samples are obtained periodically for the development of assays to detect host antibodies to the virus.

Complete necropsies are performed on any snakes that die, and tissue samples are collected for histopathology, virus isolation, and electron microscopy.

Major Findings:

The virus appears to be inducing a severe proliferative interstitial pneumonia and necrotizing interstitial pancreatitis in the kingsnakes. No viral induced lesions have yet been detected in the brown tree snakes. Paramyxovirus has been isolated from brown tree snake tissues through cell culture and identified by electron microscopy.

Significance:

Snakes and other reptiles are commonly kept in captivity, and their use as laboratory animals is increasing. Relatively little is known about the diseases of these animals. This study represents the first known attempts to experimentally transmit ophidian paramyxovirus in kingsnakes and brown tree snakes.

Proposed Course:

Continuation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-RR-10375-01 VR

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Preliminary Survey of the Incidence of Abnormal Behavior in Rhesus Monkeys

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Kathryn A.L. Bayne, M.S., Ph.D., D.V.M.

Chief, BNU

LSS, SSB, VRP, NCRR

Sandra L. Dexter, B.S., LATG

Biologist

LSS, SSB, VRP, NCRR

COOPERATING UNITS (if any)

Laboratory of Comparative Ethology, NICHD, NIH (S. Suomi)

LAB/BRANCH

Veterinary Resources Program

SECTION

Laboratory Sciences Section, SSB

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.3

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The 1985 amendment to the Animal Welfare Act (AWA) requires the provision of an environment "adequate to promote the psychological well-being" of laboratory primates. A general consensus among primatologists, veterinarians, and technical personnel is the benefit of increasing the complexity of the laboratory primate's cage to promote the animal's well-being. Recently published USDA regulations delineate methods to promulgate the AWA amendment. These methods include both social and nonsocial forms of environmental enrichment.

To meet this congressional mandate, the Laboratory of Environmental Enrichment (LEE) of the Laboratory Sciences Section has written an environmental enrichment plan for the intramural nonhuman primate program at NIH. To support the recommendations made in the NIH Nonhuman Primate Intramural Management Plan, LEE tests and implements various non-social environmental enrichment techniques including the invention of an innovative foraging device (patent pending). The success of such a program validated by some form of monitoring and evaluation process is considered by the NIH an essential component of the Primate Management Plan.

The LEE provides educational seminars on this topic. All day conferences, half-day workshops, and a series of brief presentations made in conjunction with the Office for Protection from Research Risks, and steady publication of research results are just a few examples of this educational effort.

Objective:

To assess the behavioral status of individuals from one nonhuman primate species housed in different social and physical environments by conducting a survey of abnormal behaviors in rhesus monkeys (Macaca mulatta).

Methodology:

Housing Description: The behavior of rhesus monkeys housed in three different conditions [single cage (SC), indoor/outdoor run (IO), corn crib (CC)] was evaluated. The SC condition met Guide requirements. All subjects were individually housed in this condition. Animals in the IO condition were housed in stable social groups. The indoor portion of the run was 8m², and the outdoor portion was 25m². The CC condition provided the largest amount of space (139m²). Animals were also socially housed in this condition.

Data Collection: Subjects were observed (n=15) for four, 30-minute sessions over a one month period (total=30 hours). Observations were made between 1000 and 1400 hours. A bar-code system and Tandy 102 laptop computer were used to monitor the variety, frequency and duration of abnormal behavior.

Major Findings:

All the subjects exhibited one or more patterns of abnormal behavior. Monkeys in each housing condition showed several differences in the frequency of each of the four behavior categories. A consistent pattern emerged: the greatest frequency of overall abnormal behavior and stereotypic exploratory behavior was in the SC condition, and the lowest frequency of occurrence in the CC. A similar pattern was observed in the mean duration of overall abnormal behavior and of repetitive, manipulative behavior. Notable exceptions to this pattern were that, in IO runs, the animals showed no self-directed behavior and animals in the CC condition showed the longest duration of cage manipulation.

An analysis of the four categories of abnormal behavior revealed several significant differences in behaviors in each housing condition ($p < 0.05$). The mean frequency of self-directed behaviors was significantly higher in the SC conditions than in the CC. In the frequency of stereotypic cage manipulation, there was also a significant difference between SC and CC conditions, and between SC and IO, with the SC condition consistently having higher levels. The mean duration of stereotypic behavior was significantly higher in the SC and IO conditions than in the CC condition. The mean duration of self-directed behaviors was also significantly higher in the SC condition than in the CC, while the mean duration of repetitive cage manipulation was significantly higher in the SC condition than in the IO. Also, mean duration of locomotion was significantly higher in the SC condition than in the CC.

Compared to animals in the CC condition, those in the IO showed a significantly higher ($p < 0.05$) mean percent of time per session, mean frequency of occurrence per session, and mean number of types of abnormal behaviors. No significant differences in the variables were noted between the SC and IO housing types. For the SC condition, significantly higher ($p < 0.05$) mean number of types and mean duration per session of abnormal behaviors were observed than in the CC condition, but under these conditions there was no significant difference for the mean frequency of occurrence of these behaviors.

Significance:

Abnormal behaviors that occurred significantly less in the CC condition may have been more responsive to environment modifications (greater space and social opportunities) than were abnormal behaviors which did not diminish in the various housing conditions. This possibility agrees with hypotheses about the causes of abnormal behaviors (examples of these are: an alternate form of stimulation, a means of controlling the arousal level, or a result of spatial limitations). The effect of more space on a laboratory primate's behavioral profile remains an unresolved issue. Species differences may confound these effects. No one has answered how gender and age differences affect behavior in relation to space size. Studies of closely related laboratory primates report varying degrees and directions of behavioral alteration. Greater space and social opportunities may act as buffers against other environmental factors that contribute to abnormal behaviors.

Monkeys in the SC condition showed significantly more (both longer and more frequent) self-directed behaviors than monkeys in the CC condition. This finding is consistent with the hypothesis that many abnormal behaviors are distortions of social behaviors. Thus, the social contact restriction in the SC situation may have partly caused those misdirected social behaviors (e.g., auto-aggression, self-clasp, etc.). Recent studies indicate that increase in cage space does not predictably benefit the behavioral profile; thus, periodic, compatible social partners may alleviate the severity of abnormal behaviors and decrease self-directed, abnormal behaviors.

Clearly, there is no panacea for environmental enrichment. This study showed that most abnormal behaviors occur under living conditions that preclude the full expression of species-normal behaviors. Providing environmental complexity as many researchers have proposed means providing multiple methods of behavioral stimulation from which the animals may select or retreat. Thus, it seems that environmental solutions will most likely be multifactorial.

Proposed Course:

Completed.

Publications:

Bayne K, Dexter S, Suomi S. A preliminary survey of the incidence of abnormal behavior in rhesus monkeys (Macaca mulatta) relative to housing condition. Lab Animal 1992;21(5):38-45.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-RR-10376-01 VR
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Evaluation of the Preference to and Effects of an Enriched Environment		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Kathryn A.L. Bayne, M.S., Ph.D., D.V.M.	Chief, BNU	LSS, SSB, VRP, NCRR
Joni K. Hurst, B.S.	Biologist	LSS, SSB, VRP, NCRR
Sandra L. Dexter, B.S., LATG	Biologist	LSS, SSB, VRP, NCRR
COOPERATING UNITS (if any)		
LAB/BRANCH Veterinary Resources Program		
SECTION Laboratory Sciences Section, SSB		
INSTITUTE AND LOCATION NCRR, NIH, Bethesda, MD 20892		
TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 0.5	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.) The 1985 amendment to the Animal Welfare Act (AWA) requires the provision of an environment "adequate to promote the psychological well-being" of laboratory primates. A general consensus among primatologists, veterinarians, and technical personnel is the benefit of increasing the complexity of the laboratory primate's cage to promote the animal's well-being. Recently published USDA regulations delineate methods to promulgate the AWA amendment. These methods include both social and nonsocial forms of environmental enrichment. To meet this congressional mandate, the Laboratory of Environmental Enrichment (LEE) of the Laboratory Sciences Section has written an environmental enrichment plan for the intramural nonhuman primate program at NIH. To support the recommendations made in the NIH Nonhuman Primate Intramural Management Plan, LEE tests and implements various non-social environmental enrichment techniques including the invention of an innovative foraging device (patent pending). The success of such a program validated by some form of monitoring and evaluating process is considered by the NIH an essential component of the Primate Management Plan. The LEE provides educational seminars on this topic. All day conferences, half-day workshops, and a series of brief presentations made in conjunction with the Office for Protection from Research Risks, and steady publication of research results are just a few examples of this educational effort.		

Objective:

To evaluate non-nutritive enrichments provided to laboratory primates by allowing the subjects to determine the frequency and duration of their exposure to a complex environment using a preference testing paradigm.

Methodology:

Subjects: Eight male rhesus monkeys (Macaca mulatta).

Description of Enrichment: The subjects were housed in 8.6 sq. ft. cages (two adjoining Group 3 cages). Since cage size may inconsistently have an impact on the behavioral profile of nonhuman primates, the subjects were acclimated to the double-unit cage size for 4 weeks before beginning the study. Food hoppers were placed on both sides of the double unit and the daily ration was divided equally between these hoppers to avoid inducing a cage side preference by feeding the animal on one side. No other food treats or foraging opportunities were available to the animals during the study.

A grooming board, a galvanized steel perch (Lab Products, stock #2430), a neutral color Tug-A Toy[®], and a large, red Kong[®] toy were used simultaneously to enrich one half of the cage. The grooming board was placed on the observer's upper right or left corner of the double unit on the outside of the cage. This placement expedited removal of the board for cleaning and replacement of the board's shearling. The perch was placed approximately 20 cm off the floor of the cage, parallel to the side wall. The Tug-A-Toy[®] was suspended by a short chain (6 links; 2/0 steel link coil) and connected to the inside ceiling of the cage with a 3/16" quick link, approximately 8 cm above the middle of the perch. The chain was looped through the circular portion of the Tug-A-Toy[®]. The Kong[®] toy was suspended by a 22 cm length of single-jaw steel jack-chain and attached to the outside of the cage with a double ended snap hook. The perch, Tug-A-Toy[®] and Kong[®] toy remained in place during cleaning of the cage in the cage washer. The grooming board was removed during cage cleaning and sanitized separately.

Experimental Design and Data Collection: An A-B-A Experimental design was employed. In the first baseline phase (B1), the number of occurrences in each session the subject moved into and duration of time spent in each half of the cage was recorded. Also, the frequency and duration of select behaviors in the typical repertoire of an individually housed rhesus monkey were noted. During this baseline phase, each animal was observed for eight, 30-minute sessions (two sessions per week per animal), for a total of 4 hours. During the experimental phase of the study (EXP), the four enrichment devices were placed on the side of the double unit that was used less by the monkey. As all the devices were affixed to the cage, the animal could not move the devices to the preferred cage side. Thus, each animal had to interact with the enrichment devices. The subjects' behaviors and allotment of time to each cage side were recorded for eight, 30-minute sessions during an 8-week interval. The use of each enrichment device during the EXP phase was examined by a Kruskal-Wallis analysis. The post-treatment phase of the study (B2) commenced when all enrichment devices were removed from the double unit cages. The animals were observed during this second baseline phase for eight, 30-minute sessions during a 4-week period. Again, both the behaviors of each animal and the time the subject allotted to each cage side were noted.

Major Findings:

Several significant ($p < 0.05$) behavioral findings were noted between the B1/B2 and the EXP phases. The duration of repetitive cage-directed behavior, grooming, and aberrant self-directed behavior were significantly reduced in the EXP phase when compared to both baseline phases. The duration of locomotion increased in the EXP phase when compared with the baseline phases. The

duration of stereotypic behavior was greater in the B2 phase than the B1 or EXP phases. The frequency of occurrence of repetitive cage-directed and abnormal self-directed behaviors followed the same trend as the duration data. However, no change in the frequency of grooming activity or stereotypic behavior was noted. The frequency of bouts of locomotion was significantly greater in the EXP phase only compared to B2. The subjects spent a mean of approximately 8.2 minutes, or 27% of a session interacting with the enrichment devices. No statistically significant ($p < 0.05$) decline in use of (or habituation to) the enrichment devices was detected. The subjects spent the greatest portion of the interactive time on the perch, the second greatest amount of time spent divided approximately equally between interacting with the Kong[®] and Tug-A-Toy[®], and the least amount of time spent manipulating the grooming board. Three of four animals that did not reverse their side preference also had the lowest durations of time interacting with the enrichment devices of the eight subjects. A fourth animal that did not reverse side preferences, #85170, had the highest duration of device interaction--it should be noted that this animal's stereotypy was picking at the cage in a highly focused and repetitive manner. The interaction with the enrichment device appeared to be a transference of a portion of this manipulative tendency to the devices in the cage during the EXP condition, as the time it spent in repetitive cage manipulation was reduced. Indeed, his significant increase in movement between cage sides further reflects his numerous interactions with the enrichment devices. Subjective observations further indicated that the hair coat of animals which had been self-plucking improved; attention which had been disproportionately directed to self was shifted to external factors (i.e., the enrichment devices); and new postures were observed (i.e., self-grooming while perching, wrapping an arm or leg through the Tug-A-Toy[®], bipedal standing to manipulate the grooming board or Kong[®] toy, etc.).

Significance:

Regardless of the variability in impact of enrichment on cage side preference, it is clear from the data that several changes in the animals' behavior profiles occurred concomitant with changes in their environment. During the EXP study phase, aberrant self-directed activities decreased, repetitive cage-directed activities were reduced, and excessive self-grooming was decreased. The reduction in these behaviors was considered a positive outcome of the study. Conversely, locomotor activity increased during the EXP condition. It appeared that much of this locomotion was due to the animal shifting from its B1 preferred side to the enriched cage side and back again. In summary, data show that when the enrichment is removed, the animals revert to their previous behavior habits. Thus, the positive effect(s) on behavior profile of the enrichments chosen for this study lasted only while the enrichment was present. While this may appear to be self-evident, it should be noted as a caveat to this beneficial effect, some abnormal behaviors returned at a level even more robust than initially recorded for an animal, lasting a minimum of a month at this higher level after the enrichment was removed. Indeed, the removal of the enrichment appeared to evoke a reversal of cage side preference from the EXP to B2 phases by more than one-third of the animals.

Proposed Course:

Completed.

Publications:

Bayne K, Hurst J, Dexter S. Evaluation of the preference to and behavioral effects of an enriched environment on male rhesus monkeys. *Laboratory Animal Science* 1992;42(1):38-45.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-RR-10377-01 VR			
PERIOD COVERED October 1, 1991 to September 30, 1992					
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Use of Artificial Turf as a Foraging Substrate					
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 50%; vertical-align: top;"> Kathryn A.L. Bayne, M.S., Ph.D., D.V.M. Sandra L. Dexter, B.S., LATG Hugh M. Mainzer, M.S., D.V.M. </td> <td style="width: 50%; vertical-align: top;"> Chief, BNU Biologist Veterinarian </td> <td style="width: 50%; vertical-align: top;"> LSS, SSB, VRP, NCRR LSS, SSB, VRP, NCRR PS, RAB, VRP, NCRR </td> </tr> </table>			Kathryn A.L. Bayne, M.S., Ph.D., D.V.M. Sandra L. Dexter, B.S., LATG Hugh M. Mainzer, M.S., D.V.M.	Chief, BNU Biologist Veterinarian	LSS, SSB, VRP, NCRR LSS, SSB, VRP, NCRR PS, RAB, VRP, NCRR
Kathryn A.L. Bayne, M.S., Ph.D., D.V.M. Sandra L. Dexter, B.S., LATG Hugh M. Mainzer, M.S., D.V.M.	Chief, BNU Biologist Veterinarian	LSS, SSB, VRP, NCRR LSS, SSB, VRP, NCRR PS, RAB, VRP, NCRR			
COOPERATING UNITS (if any) Division of Computer Research and Technology					
LAB/BRANCH Veterinary Resources Program					
SECTION Laboratory Sciences Section, SSB					
INSTITUTE AND LOCATION NCRR, NIH, Bethesda, MD 20892					
TOTAL STAFF YEARS: 1.5	PROFESSIONAL: 1.0	OTHER: 0.5			
CHECK APPROPRIATE BOXES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews					
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The 1985 amendment to the Animal Welfare Act (AWA) requires the provision of an environment "adequate to promote the psychological well-being" of laboratory primates. A general consensus among primatologists, veterinarians, and technical personnel is the benefit of increasing the complexity of the laboratory primate's cage to promote the animal's well-being. Recently published USDA regulations delineate methods to promulgate the AWA amendment. These methods include both social and nonsocial forms of environmental enrichment. To meet this congressional mandate, the Laboratory of Environmental Enrichment (LEE) of the Laboratory Sciences Section has written an environmental enrichment plan for the intramural nonhuman primate program at NIH. To support the recommendations made in the NIH Nonhuman Primate Intramural Management Plan, LEE tests and implements various non-social environmental enrichment techniques including the invention of an innovative foraging device (patent pending). The success of such a program validated by some form of monitoring and evaluation process is considered by the NIH an essential component of the Primate Management Plan. The LEE provides educational seminars on this topic. All day conferences, half-day workshops, and a series of brief presentations made in conjunction with the Office for Protection from Research Risks, and steady publication of research results are just a few examples of this educational effort.					

Objective:

To evaluate the effectiveness of an artificial turf foraging substrate for environmental enrichment.

Methodology:

Subjects: Eight individually housed, adult male rhesus monkeys (*Macaca mulatta*).

Description of Apparatus: The opportunity for supplemental foraging activities was provided by means of a plexiglass board (30.48 x 58.42 x 0.63 cm for the 4.3 sq. ft. cages and 35.56 x 78.74 x 0.63 cm for the 6.0 sq. ft. cages) covered with artificial turf with a turf blade length of 1.27 cm. The artificial turf was wrapped around and then bolted (3" long, 5/16" - 7.62 cm x 0.8 cm - stainless steel bolts and nuts) to the plexiglass to prevent it from being destroyed by the primates. It has a burlap backing which rhesus macaques will shred if exposed. The board was then placed inside the home cage and secured to the floor of the cage with two U-bolts and wing nuts (thereby preventing any spillage of food material from the board), one on each end of the board.

Experimental Design and Data Collection: A barcode scoring system in conjunction with a laptop computer (Tandy 102) (Line, pers comm) was used to record the behaviors of the subjects. All subjects were adapted to the presence of an observer for one week before the initiation of the study. Four 30-minute sessions of baseline data were collected for each animal over a two week period, for a total of 16 hours (PRE condition). This was followed by twenty 30-minute sessions for each subject over six months, for a total of 80 hours (EXP condition), two supplementary 30-minute sessions per animal to determine if the subjects would forage for small pieces of monkey chow (a food item of presumed lower flavor value than the Crumbles®) and four additional 30-minute sessions with no enrichment in the cage (POST condition). The order of animal observation was randomized and balanced between conditions. Data were collected between 1000 and 1400 hours.

Major Findings:

The animals displayed a minimum of four different types of aberrant behavior during the initial baseline sessions, which did not necessarily include behaviors from all categories of abnormal behavior. The overall analysis of the total of the four categories of behaviors demonstrated that in the EXP condition, duration and frequency of these behaviors were significantly lower than the PRE condition ($p < 0.002$, $p < 0.0001$) and POST condition ($p < 0.0001$, $p < 0.0001$) with no evidence for a difference between the PRE and POST conditions. Specifically, the repetitive locomotion category was significantly reduced in the EXP condition as compared to the PRE condition (duration: $p < 0.0004$, frequency: $p < 0.0001$) and POST condition (duration: $p < 0.0004$, frequency: $p < 0.0004$). Self-directed behavior was higher in duration and frequency in the POST condition than the EXP condition ($p < 0.003$, $p < 0.005$). Also, the duration of stereotypic behavior was significantly reduced when a foraging opportunity was available in the EXP condition ($p < 0.05$). There was no evidence to support that cage-directed behavior in the EXP condition was lower in either duration or frequency than in the PRE or POST conditions.

Significance:

The use of artificial turf as a foraging substrate made this enrichment device in many ways easier to manage than the fleece substrate described by Bayne et al. Although both substrates resulted in significant reductions in abnormal behavior, and with both devices the subjects showed increased usage of the enrichment technique (a mean use over time of approximately 52% for artificial turf and 40% for fleece), the artificial turf proved to be quicker to re-load with particulate food (as it did not have to be detached from the cage first). It also was not necessary to remove the board from the cage for cleaning purposes (as was the fleece board). However, unlike the fleece board, no grooming activities directed at the board were noted and no social signals were directed to the turf board. One possible disadvantage to the artificial turf substrate is related to its positioning on or under the cage floor.

Proposed Course:

Completed.

Publications:

Bayne K, Dexter S, Mainzer H, McCully C, Campbell G, Yamada F. The use of artificial turf as a foraging substrate for individually housed rhesus monkeys (Macaca mulatta). Animal Welfare 1992;1:39-53.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-RR-10400-01 VR

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Idiopathic Hepatitis in the N:NIH(S)-B Mouse

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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COOPERATING UNITS (if any)

Genetic Resource Section, SSB, VRP, NCRR

LAB/BRANCH

Veterinary Resources Program

SECTION

Laboratory Sciences Section

INSTITUTE AND LOCATION

NCRR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.05

PROFESSIONAL:

0.025

OTHER:

0.025

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

All N:NIH(S)-B mice examined have multiple foci of hepatic necrosis and inflammation. The cause of hepatitis is unknown. This strain was developed at the NIH Animal Genetic Resource for use as foster mothers in the barrier facility. All mice appear clinically normal, but have significant abnormalities in their livers. Numerous studies are in progress to determine the etiology and explore the pathogenesis of hepatitis in this strain.

Many mice have numerous white foci and depressions visible on liver surfaces. Microscopic foci of hepatitis and coagulation necrosis are present in all mice. Transmission EM has failed to detect any pathogens. Mouse hepatitis virus antigen is not present using immunochemistry. Lymphocyte and macrophage cell markers will be used to explore pathogenesis and identify the origin of multinucleate syncytial cells in lesions. No viruses or bacteria have yet been detected. Many mice have elevated serum enzymes indicating hepatocellular necrosis. Oral and parenteral inoculation of SCID mice with fresh homogenized affected livers failed to result in transmission. Vitamin A is being examined as a possible etiology or co-factor. Mouse strains closely related to the N:NIH(S)-B are being examined for hepatic abnormalities.

The cause of hepatic necrosis and inflammation in the N:NIH(S)-B mouse remains unknown. An infectious etiology is unlikely. Genetic factors appear important. Hepatitis in this mouse strain may serve as a useful model to study the pathogenesis of hepatitis in animals and humans.

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